Genetic structure analysis and population assignment tests to determine differential natural productivity among hatchery-origin and natural-origin steelhead trout (*Oncorhynchus mykiss*) in Eagle Creek, OR.

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Prepared by: Andrew P. Matala and William Ardren,
Applied Research Program in Conservation Genetics
Abernathy Fish Technology Center
U.S. Fish and Wildlife Service
Longview, WA 98632
(360)425-6072x256
andrew_matala@fws.gov
william_ardren@fws.gov

In cooperation with:
Doug Olson, Maureen Kavanagh, Bill Brignon, Jeff Hogle
Hatchery Assessment Team
Columbia River Fisheries Program Office
U.S. Fish and Wildlife Service
Vancouver, WA 98683

INTRODUCTION

Hatchery operation and description of Eagle Creek populations

Propagation of steelhead trout (*Oncorhynchus mykiss*) at Eagle Creek National Fish Hatchery (ECNFH) was implemented to mitigate for loss of fishery resources in the Columbia River basin resulting from construction of dams. Steelhead production at ECNFH also contributes to commercial, sport, and tribal harvests in the region (Eagle Creek Hatchery and Genetic Management Plan). Steelhead smolts reared at ECNFH are volitionally released into Eagle Creek within the Clackamas River basin (Figure 1). On-station releases of one-year-old smolts averaged 176,000 per year (range 113,000 – 207,000) over the period 1990-2002. Over the years 1980 to 2002, the annual return of ECNFH-origin steelhead to Eagle Creek averaged 805, and ranged from 251 to 3,671.

The original ECNFH brood stock was derived from a mix of native Clackamas winter-run steelhead stocks, and stock from the Big Creek Hatchery located in the lower Columbia River. The Big Creek winter-run brood stock is characterized by a high survival rate in the hatchery, and early run-time and spawn-time. Hatchery-origin (HAT) steelhead in Eagle Creek return from November through April, and the largest contingent returns between December and mid March. Spawning among later returning natural-origin (NOR) steelhead in Eagle Creek typically begins in April, and is completed by mid-June, with peak activity in May (ODFW 1992). Some overlap in return time between HAT and NOR has been observed. Differential spawning times minimize the opportunity for interbreeding and natural introgression between HAT and NOR steelhead, and the temporal distinction allows for targeted HAT steelhead harvests with minimal impact on the NOR counterpart. The current protocol for the steelhead hatchery program at ECNFH was established in 1992. Operations involve annual collection of a fully segregated brood stock, taken exclusively from among steelhead captured at the hatchery rack with an identifiable ECNFH mark.

Eagle Creek population concerns and status

The NOR winter steelhead in Eagle Creek are among populations listed as threatened under the Endangered Species Act (ESA; Lower Columbia River ESU, 63 FR 13347; March 19, 1998). Although the majority of NOR steelhead production in the Clackamas River system occurs in the upper Clackamas River (upstream of North Fork Dam), the Eagle Creek watershed is responsible for a valuable proportion of overall production. In the Eagle Creek watershed, NOR steelhead are believed to spawn primarily in the North fork, but some natural spawning is also thought to occur in the lower 0.3 miles of Bear Creek, the lower 2 miles of Little Eagle Creek, Delph Creek and the main stem of Eagle Creek downstream of the hatchery.

The ESA listing of Columbia River steelhead led to a subsequent ruling stating that the use of out-of-basin brood stocks in hatcheries jeopardizes ESA-listed NOR populations in the same watershed. Prompted by this ruling, a contract (FWS Agreement # H012A) was established between USFWS, NMFS-Northwest Fisheries Science Center (NMFS) and Washington Department of Fisheries and Wildlife (WDFW) to conduct a genetic evaluation. In this evaluation, the level of genetic similarity among four steelhead populations was described, and included, ECNFH brood stock, Clackamas River NOR "late run", Eagle Creek NOR "late run" and Big Creek HAT brood stock. In a preliminary analysis, significant allele frequency differences were observed among all four populations (pers. comm. Don Campton, USFWS). The two NOR "late run" populations were more genetically similar to one another than were the hatchery populations. The ECNFH population was most genetically similar to the Big Creek hatchery and Native Eagle Creek "late run" populations. Overall, these results suggest that the ECNFH brood stock and Big Creek stock have become genetically introgressed, resulting in a relatively large genetic difference between the NOR Eagle Creek late-run population and the ECNFH brood stock. The genetic evaluation further demonstrated very low levels of gene flow between ECNFH brood stock and NOR Eagle Creek fish despite the opportunity for both populations to spawn naturally in Eagle Creek below the hatchery.

Natural productivity in Eagle Creek: HAT vs. NOR steelhead

Return and spawn timing differences between HAT and NOR steelhead in Eagle Creek suggest these two groups have low rates of genetic exchange. The potential for gene flow between the groups is also reduced by spatial segregation among respective spawning locations. Observational and tagging evidence indicates that late run NOR steelhead primarily spawn in the North Fork Eagle Creek and that the majority of HAT fish that spawn naturally, do so in the main stem of Eagle Creek below the hatchery

Several studies have documented low lifetime natural reproductive success of hatchery origin steelhead trout when they originate from out-of-basin multigenerational hatchery programs (Waples 1999, Araki and Ardren 2006). Progeny (0+) of naturally spawning HAT steelhead may have high survival through their first year, but with few reaching the smolt stage (Chilcote et al. 1986), and subsequent low escapement. When this occurs, the increased competition among young-of-the-year fish (i.e. over-wintering) will negatively effect overall NOR survival, without the benefit of a demographic boost in the naturally spawning population contributed by HAT steelhead. Moreover, less fit naturally spawning HAT steelhead may put the NOR population at risk in Eagle Creek when HAT and NOR steelhead interbreed.

In another component of this study, the USFWS Columbia River Fisheries Program Office is collecting data on run timing, behavior, distribution, and abundance of hatchery and wild steelhead in Eagle Creek (Kavanagh et al 2006). In addition, the USFWS Lower Columbia River Fish Health Center is collecting information on fish health and disease status of wild and hatchery fish in Eagle Creek. Together, these investigations will provide a better understanding of the ecological interactions between hatchery and wild fish and ultimately help improve our hatchery operations in the context of watershed management

Study Objectives

This report covers the second year of an ongoing, long-term study. In this second year we focused on the same questions as were addressed in the previous year, but with an additional explanation of the level of temporal variation observed for results among years. We used population genetic structure analyses and assignment tests to evaluate the level of gene flow and relative natural productivity of NOR and HAT steelhead within the Eagle Creek watershed. We evaluated possible spatial variation in spawning and rearing habitats utilized by NOR and HAT steelhead. The null hypothesis tested was:

H_o: no difference in natural productivity among hatchery-origin (ECNFH) and natural-origin steelhead in the Eagle Creek watershed.

In subsequent years we will also describe the relationship between other Clackamas River NOR and HAT steelhead populations, and Eagle Creek NOR and HAT populations. Our goal in this effort will be to determine how stray steelhead in Eagle Creek may influence the characterization of populations and natural productivity of steelhead trout throughout the watershed. Microsatellite genotypic data for Clackamas River steelhead will be collected and provided by Paul Moran and Maureen Waite from NOAA Fisheries.

METHODS

Collection of Samples

Genetic samples were collected by the Columbia River Fisheries Program Office and Lower Columbia River Fish Health Center. Four steelhead sample groups comprised of juveniles and/or smolts were targeted (Figure 1) with a goal of 50 *O. mykiss* from each location. Group one samples were collected within the section extending from the Eagle Creek confluence with the Clackamas River, upstream to the North Fork Eagle Creek confluence (Lower E. C.). Group two samples were collected within the North Fork Eagle Creek (N. Fork E. C.). The third group was collected within the section that begins at the confluence of Eagle Creek and North Fork Eagle Creek, and extends upstream to the ECNFH (Upper E. C.). The last group was collected directly from the raceways at ECNFH. Adult-NOR returning to Eagle Creek were sampled from the lower ladder in the main stem.

Sample summary for FY2006

Reach/location	Origin	Target (n)	Juvenile (n)	Smolts (n)	Total (n)
1.) Lower E. C.	NOR	50	26	3	*29
2.) N. Fork E. C.	NOR	50	27	42	69
3.) Upper E. C.	NOR	50	69	25	94
4.) Eagle Creek-NFH	HAT	50	0	48	*48
5.) Lower Ladder	Adult NOR	50	----	----	*29
**did not meet target*		250	122	118	269

Sampling in the N. Fork E. C. was done in conjunction with regular screw trap operation by the U. S. Forest Service. Because too few smolts were encountered during collections, it was necessary to include juvenile (1+) *O. mykiss*, which were sampled using electroshock methods. With the exception of screw traps, sample collections were distributed throughout each section to avoid sampling siblings or family groups, and include: the area adjacent to Eagle Fern Park, immediately below the confluence of Eagle Creek and North Fork Eagle Creek, below the main stem lower ladder, and directly above the confluence of Eagle Creek and the Clackamas River.

Biological data including fork length and weight measurements were recorded during collection of each sample (Appendix 1), and scales were taken for age determination from a subset of (0+) juveniles. Adult NOR were sampled in coordination with radio tagging captures at the Eagle Creek main stem lower ladder. Fork lengths were recorded for each adult fish, and scales were taken for age determination. A small piece of fin tissue was removed from each fish sampled, and these were placed in individual vials of 100% non-denatured EtOH, and labeled with a unique identification number. Vials were sent to the Conservation Genetics Program Laboratory at Abernathy Fish Technology Center for DNA extraction and analysis.

Microsatellite Amplification and Analysis

(Note: for complete detailed methods, see FY05 Results Summary:FONS# 13210-2005-011, Dec. 2005.)

The following 16 microsatellite (nuclear DNA loci) primers were amplified: *μOmy1011UW* (Spies *et al.* 2005), *μSsa407* and *μSsa408* (Cairney *et al.* 2000), *μOne13* and *μOne14* (Scribner *et al.* 1996), *μOcl1* (Condrey & Bentzen 1998), *μOgo4* and *μOgo3* (Olsen *et al.* 1998), *μOts4, μOts100, μOts3 and μOts1* (Banks *et al.* 1999), *μOki23* (Smith *et al.* 1998), *μOmy7iNRA* (K. Gharbi, and R. Guyomard, Unpublished), *μOmy77* (Morris *et al.* 1996), and *μSsa289* (McConnell 1995). This is the same suite of markers evaluated in the previous year.

A pairwise genetic distance matrix of Cavalli-Sforza and Edwards (1967) chord distances (CSE) was generated using the software program PHYLIP version 3.5C (Felsenstein 1992). The neighbor-joining phylogram topology was constructed based on pairwise genetic distances for all available data to date (i.e. both 2005 and 2006 sample collections). Each temporal sample group is displayed uniquely.

Factorial correspondence (FC) analysis of individual multilocus scores was conducted using GENETIX version 4.05 (Belkhir *et al.* 2004). Correlations among groups were evaluated in five comparisons: ECNFH vs. adult NOR, ECNFH vs. upper E.C., ECNFH vs. lower E.C., ECNFH vs. N. Fork E.C., and the spatial relationship among all NOR groups.

Species ID: hybrid screening

In reaches of the Eagle Creek watershed where coastal cutthroat trout (*O. clarkii*) are present, hybridization with steelhead trout may occur. In other regional watersheds where both species co-occur, hybrid individuals have been observed with intermediate physical traits (Erik Olsen, ODFW, personal communication). We confirmed the species ID (or F1 hybrid identity) of all juveniles sampled for the Eagle Creek analysis that were identified phenotypically as *O. mykiss*.

We used the bi-parentally inherited, species-specific marker OM-42 (Ostberg and Rodriguez 2004). Sample DNA was amplified under the same conditions as described for all other loci, but with an annealing temperature of 60^{o}C. PCR product sizes were visualized on 3.5% agarose gels stained with ethidium bromide, and standardized with Hi-Lo™ DNA Marker (Minnesota Molecular Inc.).

Assignment tests: HAT vs. NOR

Hatchery smolts from ECNFH and the adult-NOR samples collected at the main stem lower ladder represent steelhead of “known” origin, from which the HAT/NOR genotypic data baseline was constructed. The baseline is an allele frequency standard against which all unknown samples were measured. Origin of individuals in each sample group was determined based on similarity of genotype to one or the other of the population allele frequencies in the baseline. The juvenile groups from the four reaches within the Eagle Creek watershed were treated as fish of “unknown” origin. In the FY2006 analysis we combined ECNFH and adult NOR samples from both the 2005 and 2006 sample years to construct the baseline. This practice is standard in assignment analyses, and adjusts for temporal variability in allele frequencies within baseline populations that may otherwise bias results. The plot of equal probability (Figure 6) was constructed using the absolute value of log transformed likelihood values.

RESULTS

Descriptive Statistics

There was a high level of variability or allelic polymorphism among 16 loci evaluated in the 2006 Eagle Creek steelhead dataset. Numbers of alleles ranged from 5 at *μSsa289* in the main stem groups, to 19 at *μSsa408* in the N. fork E. C. group (mean = 11 over loci and groups). Observed heterozygosity ranged from 0.51 at *μOts4* in the upper E. C. group, to 0.96 at *μSsa408* in the adult NOR group (mean = 0.76 over loci and groups). We observed one departure from expected genotypic proportions within groups (HWE), at *μOcl1* in the ECNFH group. There was no indication of heterozygote deficit; a deficit could mean the presence of "null" allele or large allele dropout. The number of private alleles among groups ranged from 1 in the lower E. C. group to 11 in the upper E. C. group (Table 1), and a significant difference in allelic richness ($P < 0.04$; Figure 2) was observed in the comparison of the ECNFH group to all remaining (NOR) groups.

Population Genetic Structure Analysis

Population structure was observed among the 5 groups of steelhead evaluated in the 2006 Eagle Creek study. The F_{ST} values ranged from 0.000 to 0.029 among loci (Table 1), and the overall estimate of 0.009 falls within the 95% confidence interval for significance (0.006-0.013). The tests of population homogeneity (H_o: no difference in allele frequencies among groups), indicate restricted gene flow (heterogeneity) among NOR and ECNFH groups. The number of observed locus specific differences ranged from 6 of 16 loci in the ECNFH vs. adult NOR and ECNFH vs. lower E.C. tests, to 11 of 16 loci in the ECNFH vs. N. fork E.C. test (Table 2). With the exception of *μOmy7i* in the adult NOR vs. upper E.C. test, there was no indication of significant population differentiation between the adult-NOR group and all remaining NOR (juvenile) groups (Table 2).

Population genetic structure and significant heterogeneity among ECNFH and NOR groups is corroborated by the relationship of pairwise genetic distances demonstrated in the topology of the NJ phylogram (Figure 3). The ECNFH groups from both 2005 and 2006 cluster closely together, and the bootstrap support (>99%) suggests they are genetically distant from all NOR groups. The greatest similarity (also with high bootstrap support) among NOR groups is seen on the branch shared by the 2005 upper and lower E.C. sample groups. In addition, the 2006 upper E.C. group was observed clustering closely with the 2005 adult-NOR group.

The relationship among ECNFH and NOR groups is further explained using FC plots showing spatially arrayed maximum variability (Figure 4). The variation among the lower E. C. and ECNFH groups is well defined with some overlap. The upper E. C. and ECNFH group comparison shows a recognizable separation of data clusters, but maximum variability (discreteness) is not as large as was observed in the 2005 analysis. The plot of ECNFH and adult-NOR groups appears to have less variation but maintains a similarly defined separation of groups with an area of overlap. Although the N. Fork E. C. and ECNFH group comparison in 2005 appeared to suggest the least amount of variation among groups, the results of the 2006 analysis indicate a larger maximum variability and more defined relationship. In comparison, maximum variability among all NOR groups is small and the data appear to form a single cluster, indicating greater similarity among groups.

Evidence of hybridization and misidentification

Hybrid screening was conducted using genetic markers that amplify species-specific DNA fragments. We detected three *O. clarki / O. mykiss* F1-hybrid individuals in the N. fork E.C. group. These three individuals exhibited heterozygous genotypes. We detected one misidentified coastal cutthroat trout in the upper E.C. group and one misidentified coastal cutthroat trout in the ECNFH group. These individuals exhibited homozygous cutthroat genotypes. All hybrid and cutthroat samples were omitted from the dataset and from all analyses.

Baseline assignment tests

The overall assignment power is defined as the proportion of baseline individuals correctly assigned to group-of-origin in the jackknife re-sampling procedure. Individuals in the ECNFH group assigned to their group-of-origin with 91.3% accuracy (LOD < 0), while the adult-NOR in the baseline assigned to their group-of-origin with 76.1% accuracy (LOD > 0). Within the ECNFH group, 95% assignment confidence required a score of LOD < -1.87. Within the adult-NOR group, 95% assignment confidence required a score of LOD > 1.39. Only 54.8% of ECNFH and 53.5% adult-NOR fish in the baseline met these requirements (Table 3). The LOD bounds required for 95% confidence (expanded beyond zero) are a reflection of the proportion of mis-assigned individuals (i.e. known ECNFH scoring LOD > 0, or known adult-NOR scoring LOD < 0) seen in the overlapping distribution of baseline LOD scores (Figure 5).

Assigning HAT or NOR origin to "unknown" groups

Among the 2006 samples, the proportion of NOR assignments was comparable for lower, upper and N. fork Eagle Creek groups (79.3%, 80.9, and 82.8% respectively), although at the 95% confidence limit these proportions decreased by approximately 20% (Table 3a). The proportion of HAT assignments meeting the 95% confidence limit was considerably smaller for all groups; the largest proportion of HAT assignments (14.5%) was observed within the N. fork E.C. group. These assignment results can be seen graphically as relative assignment likelihood values (HAT/NOR) for all 2006 sample groups, using the method of Hendry et al. (2002; Figure 6). In this plot, fish that fall on the line have an equal probability of HAT or NOR assignment (LOD score = 0). Finally, both the 2005 and 2006 samples were combined for each "unknown" group respectively, and assignments were recalculated. The percentage of NOR and HAT assignments among the three groups were similar to proportions observed among only the 2006 samples; however, the proportion of individuals that assigned to NOR or HAT origin at the 95% confidence level changed significantly in nearly every case. For example, we observed 58% NOR assignment (95% confidence) in the 2006 N. fork E. C. group, but when the N. fork E. C. group also included samples from 2005 the proportion of NOR assignments overall dropped to 33.1% (Table 3b). There were a surprisingly large number of alleles observed among all "unknown" groups in 2006 (relative to sample size) that were not observed in the collective baseline (2005 and 2006). Most "new" alleles (n = 42) were associated with individuals that were assigned NOR origin, while only three "new" alleles were associated with ECNFH assignments (Table 3a, 3b).

DISCUSSION

Previous studies (USFWS unpublished) have confirmed that the ECNFH brood stock has undergone genetic introgression with out-of-basin Big Creek steelhead, and is considered a relatively early returning stock. Native Clackamas River and Eagle Creek NOR stocks are typically considered late-run steelhead. Genetic differences or restricted gene flow between the two groups is largely the result of this temporal separation in run and spawn time. Hatchery brood stocks are collected exclusively from HAT steelhead returning to the hatchery, a practice that helps to maintain the discreteness of ESA-listed NOR fish and the temporal separation of the stocks. However, there is concern that ECNFH steelhead that spawn naturally below the hatchery could be impacting the survival of Eagle Creek NOR steelhead. In this study we have addressed the principal, overarching question; do hatchery and natural-origin steelhead contribute equally to the production of natural origin steelhead trout in Eagle Creek? If so how is production spatially distributed within the watershed?

The results in 2006 are complimentary to those reported in the 2005 study, particularly in regard to comparisons of pairwise genetic distance and homogeneity tests, the latter of which suggest that ECNFH steelhead do not reproduce successfully in the wild, or contribute very little to natural production of (1+) progeny in the Eagle Creek watershed. Based on locus-specific significance tests, marked genetic differences were observed between the ECNFH stock and juveniles collected from locations within the N. fork and upper reaches of Eagle Creek. A significant but less pronounced difference was observed between the ECNFH stock and both the lower E.C. and adult-NOR groups. In contrast, we did not observe any evidence of restricted gene flow that would indicate reproductive isolation between the adult-NOR group and the lower, upper, and N. fork Eagle Creek juvenile groups.

The results of assignment tests appear to strengthen the conclusion that ECNFH are genetically different from the NOR groups (recall approx. 92% ECNFH assignment accuracy in the jackknife procedure), but overall the interpretation of these results in regard to similarities or differences among the groups is considerably more ambiguous. When displayed graphically (Figures 5 and 6), assignment likelihood values among HAT and NOR groups do not appear to be distributed randomly. However, the current combination of loci and baseline samples provides relatively low assignment power, and would not be adequate to differentiate between individuals for the purpose of making group distinctions (Matala *et al.* 2005). The most likely reason for the lack of resolving power can be linked to small sample sizes and the large number of alleles among "unknown" groups that were not observed in the baseline.

Although different, it is not surprising that ECNFH and NOR groups are not distinct given that the original brood stock for the ECNFH included a significant proportion from native Clackamas River and Eagle Creek stocks. Brood stock origin notwithstanding, the 2006 results do support the conclusion that there is little natural production of HAT steelhead in the main stem of Eagle Creek, despite the opportunity to do so. One explanation is that earlier returning HAT steelhead reach the hatchery before biological or environmental queues to spawn are "switched on". However, HAT steelhead that stray into the North Fork Eagle Creek, remain for extended periods, and are not intercepted (as at the hatchery) may attempt to spawn at some point.

Because of the temporal separation of the NOR and HAT winter steelhead in Eagle Creek it is reasonable to infer that most naturally spawning HAT will spawn with other HAT steelhead. If HAT adult are reproducing successfully (producing viable progeny), it appears the effect is not realized among older (1+) juveniles or the adult population. The adult NOR were observed to be genetically different from the ECNFH group, but genetically similar to all three juvenile sample groups collected within Eagle Creek. It is likely that the combination of an early return and earlier natural spawning time among HAT fish contributes to lower survival rates and decreased fitness among their naturally produced progeny. These progeny may have mal-adapted incubation periods or emergence times in comparison to progeny of later spawners, and may be forced to contend with decreased food availability and water temperatures that are sub-optimal.

In contrast to these results, Kostow *et al.* (2003) observed a substantial contribution to natural smolt production from HAT summer steelhead in the Clackamas River Basin. However, introduced summer steelhead and co-occurring winter steelhead have different life histories (i.e. run timing vs. spawn timing) that may contribute to higher levels of introgression among the two steelhead populations. Similar to our results, McLean *et al.* (2004) observed differential reproductive success among sympatric groups of HAT and NOR steelhead, with evidence of relatively poor natural production by HAT adults; assignment success of unknown fish was as high as 82%, and natural production of smolts by HAT females was only 4.4 -7.0 % that of NOR females (see also Chilcote et al. 1986).

Throughout these analyses, we have observed several results, including significant allelic richness between NOR and HAT, large number of private alleles, and a large proportion of new alleles in assignment tests, that indicate insufficient sampling. Continued temporal sampling and an expansion of the baseline will provide a better characterization of the true population allelic distribution among NOR and HAT Eagle Creek steelhead. It is interesting that despite the number of new alleles observed among "unknown" samples, the overwhelming majority of assignments were of NOR origin. The genetic assignment method does not necessarily distinguish crossbred, or out-of-basin stray steelhead trout; rather, similarity of multilocus genotypes between the baseline populations and the individual being evaluated provide an unequivocal assignment regardless of a fish's true origin; in other words, an assignment is forced even if the individual is not a true member of either baseline population. If cutthroat trout hybridization is more pervasive than realized, including the rate of backcrossing, or Clackamas River NOR strays are reproducing in Eagle Creek, it would be necessary to have this information in order to make accurate conclusions about the true population structure of native Eagle Creek steelhead. We intend to explore these concerns in subsequent years by continued hybrid screening, and inclusion of Clackamas River NOR steelhead data in our analyses.

Evidence to date supports current ECNFH hatchery protocol and operation, at least in the short-term. Although ECNFH brood stock origin has a component of out-of-basin hatchery stock, HAT return is temporally segregated, and does not appear to pose a threat to the genetic integrity of late-returning NOR Eagle Creek steelhead. However, a further understanding of temporal trends or changes in reproductive success of Eagle Creek steelhead is essential, and will provide additional incite into differential natural production and survival of NOR and HAT. In the future, we suggest including additional sections from above the fish barrier and from Delph and Bear Creeks in the analysis, to benefit both the temporal and spatial genetic characterization of *O. mykiss* throughout the Eagle Creek watershed.

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Table 1. Descriptive statistics for the 2006 Eagle Creek Steelhead population structure analysis. Column headings are defined as follows: **n** is the number of individuals, **A** is the number of alleles, **AR** is the allelic richness, **AP** is the number of private alleles, $\mathbf{H_E}$ is Nei's (1978) unbiased estimate of expected heterozygosity, $\mathbf{H_O}$ is the observed heterozygosity, $\mathbf{F_{is}}$ in the index of inbreeding, and $\boldsymbol{\theta}$ is the unbiased estimate of Wright's F_{ST} (Weir and Cockerham 1984). Bold values with the symbol (*****) indicate statistical significance (Rice 1989; $\alpha = 0.05$ adjusted by α/k).

	Upper E. C. - 25 smolts, 72 Juv.							Lower E. C. - 3 smolts, 26 Juv.						
Locus	n	A	AR	AP	H_E	H_O	F_{is}	n	A	AR	AP	H_E	H_O	F_{is}
µOne13	93	18	11.5	3	0.831	0.860	-0.035	29	12	11.5	0	0.854	0.828	0.032
µOne14	94	10	7.2	1	0.775	0.777	-0.002	28	8	7.9	0	0.773	0.714	0.078
µOgo3	94	8	5.5	2	0.629	0.596	0.052	29	5	4.9	0	0.583	0.621	-0.067
µOki23	94	13	10.7	1	0.840	0.872	-0.038	29	11	10.7	0	0.857	0.828	0.034
µOmy1011	94	15	11.3	1	0.866	0.872	-0.008	29	13	12.8	0	0.903	0.931	-0.037
µOmy77	94	13	10.5	0	0.870	0.872	-0.003	29	13	12.7	0	0.873	0.897	-0.028
µSsa289	94	5	4.3	0	0.598	0.606	-0.014	29	5	5.0	0	0.644	0.586	0.067
µSsa407	94	16	11.8	1	0.845	0.787	0.067	29	10	9.6	0	0.740	0.828	-0.120
µSsa408	93	16	13.8	0	0.913	0.957	-0.048	29	14	13.8	0	0.913	0.931	-0.020
µOcl1	94	13	11.2	0	0.836	0.755	0.096	29	11	10.7	0	0.798	0.793	0.006
µOgo4	94	8	7.2	0	0.811	0.809	-0.004	29	7	7.0	0	0.812	0.828	-0.019
µOmy7i	94	13	10.8	0	0.832	0.872	-0.049	29	10	9.7	0	0.693	0.724	-0.046
µOts1	94	12	8.5	0	0.680	0.681	-0.003	29	11	10.7	0	0.745	0.621	0.170
µOts100	93	15	11.4	2	0.882	0.892	-0.012	28	13	12.8	1	0.895	0.857	0.041
µOts3	94	9	6.5	0	0.679	0.574	0.155	29	8	7.7	0	0.731	0.621	0.142
µOts4	94	7	5.7	0	0.511	0.511	-0.014	29	7	6.9	0	0.548	0.552	-0.007
Over All	--	--	--	***11***	--	--	--	--	--	--	***1***	--	--	--

	N. Fork EC - 43 smolts, 31 Juv.							ECNFH - 48 smolts						
Locus	n	A	AR	AP	H_E	H_O	F_{is}	n	A	AR	AP	H_E	H_O	F_{is}
μOne13	70	17	12.4	0	0.866	0.857	0.010	48	12	9.9	1	0.807	0.708	0.116
μOne14	70	9	6.7	0	0.757	0.671	0.106	48	9	8.2	0	0.798	0.875	-0.104
μOgo3	70	7	6.1	1	0.624	0.557	0.103	48	6	5.3	0	0.649	0.583	0.102
μOki23	70	12	9.2	0	0.831	0.843	-0.015	48	11	10.3	0	0.896	0.917	-0.023
μOmy1011	70	17	12.4	0	0.875	0.900	-0.028	48	14	12.4	0	0.874	0.833	0.047
μOmy77	70	14	11.4	1	0.867	0.829	0.045	47	11	10.0	0	0.848	0.872	-0.029
μSsa289	70	6	5.6	1	0.634	0.657	-0.036	48	6	5.5	0	0.618	0.688	-0.113
μSsa407	70	15	11.6	1	0.860	0.871	-0.014	48	13	11.2	0	0.867	0.875	-0.009
μSsa408	70	19	15.9	2	0.921	0.871	0.054	48	14	12.5	0	0.874	0.875	-0.001
μOcl1	70	15	11.9	2	0.826	0.829	-0.003	48	13	12.1	0	0.891	0.729	**0.183***
μOgo4	70	9	8.3	0	0.853	0.814	0.045	48	9	7.6	0	0.773	0.729	0.050
μOmy7i	70	13	10.1	0	0.744	0.743	-0.008	48	10	8.3	0	0.740	0.813	-0.099
μOts1	70	9	7.4	0	0.655	0.571	0.128	48	10	9.4	0	0.757	0.708	0.053
μOts100	70	14	11.4	2	0.894	0.886	0.007	48	12	11.1	1	0.890	0.896	-0.006
μOts3	70	7	5.3	0	0.614	0.529	0.129	48	6	5.7	0	0.620	0.625	-0.029
μOts4	70	7	6.6	0	0.590	0.629	-0.083	48	7	6.4	1	0.568	0.542	0.048
Over All	--	--	--	***10***	--	--	--	--	--	--	***3***	--	--	--

	NOR – 29 adult steelhead							Mean							
Locus	n	A	AR	AP	H_E	H_O	F_{is}	n	A	AR	AP	H_E	H_O	F_{is}	F_{st} (θ)
µOne13	29	14	13.5	0	0.864	0.897	-0.042	53.8	14.6	12	0.8	0.842	0.830	0.015	0.029
µOne14	29	7	6.9	0	0.786	0.655	0.158	53.8	8.6	7.6	0.2	0.774	0.739	0.046	0.001
µOgo3	29	6	5.9	1	0.64	0.724	-0.168	54.0	6.4	5.7	0.8	0.620	0.616	0.007	0.012
µOki23	29	11	10.8	0	0.842	0.828	0.018	54.0	11.6	10.7	0.2	0.853	0.857	-0.005	0.01
µOmy1011	29	13	12.8	0	0.901	0.931	-0.034	54.0	14.4	12.8	0.2	0.883	0.894	-0.012	0.005
µOmy77	28	13	12.6	0	0.888	0.893	-0.007	53.6	12.8	11.7	0.2	0.869	0.873	-0.004	0.011
µSsa289	29	6	5.9	0	0.616	0.517	0.163	54.0	5.6	5.1	0.2	0.619	0.611	0.013	0.012
µSsa407	29	12	11.5	1	0.828	0.828	0.001	54.0	13.2	12.1	0.6	0.828	0.838	-0.012	0.007
µSsa408	28	15	14.8	0	0.930	0.964	-0.038	53.6	15.6	14.8	0.4	0.910	0.920	-0.011	0.006
µOcl1	26	12	12.0	0	0.887	0.808	0.089	53.4	12.8	12	0.4	0.847	0.783	0.077	0.000
µOgo4	29	7	7.0	0	0.768	0.655	0.149	54.0	8.0	7.9	0.0	0.801	0.767	0.043	0.004
µOmy7i	28	10	9.8	1	0.759	0.607	0.203	53.8	11.2	10.5	0.2	0.752	0.752	0.000	0.002
µOts1	29	12	11.6	1	0.754	0.69	0.087	54.0	10.8	9.3	0.2	0.716	0.654	0.087	0.002
µOts100	29	12	11.5	1	0.845	0.897	-0.077	53.6	13.2	11.9	1.4	0.878	0.886	-0.008	0.002
µOts3	29	8	7.9	0	0.699	0.828	-0.188	54.0	7.6	6.6	0.0	0.663	0.635	0.042	0.022
µOts4	28	7	6.9	0	0.701	0.786	-0.153	53.8	7.0	6.5	0.2	0.577	0.604	-0.047	0.012
Over All	--	--	--	***5***	--	--	--	53.8	10.8	9.8	0.4	0.783	0.765	--	**0.009***
L95% CI	--	--	--	--	--	--	--	--	--	--	--		--	--	*0.006*
U95% CI	--	--	--	--	--	--	--	--	--	--	--		--	--	*0.013*

Table 2. Monte Carlo chi-square tests of homogeneity (Zaykin and Pudovkin 1993). The procedure tests the null hypothesis – H_o: no difference in allele frequencies among HOR and NOR groups. Bootstrap probabilities (*P*) were derived from 50,000 simulated random samples. Significant heterogeneity is shown in bold type: $P < 0.01$(**), and $P < 0.001$(***). Statistical significance (α) has been adjusted for the number of simultaneous tests *k* (α/k for $\alpha = 0.05$) by the sequential Bonferroni correction (Rice 1989). See methods section for descriptions of Eagle Creek steelhead groups.

A.) Adult NOR vs. ECNFH				B.) N. Fork E.C. vs. ECNFH				C.) N. Fork E.C. vs. Adult NOR			
Locus	df	χ^2	*P* - value	Locus	df	χ^2	*P* - value	Locus	df	χ^2	*P* - value
μOmy77	13	35.448	***	*μOcl1*	15	60.572	***	*μOgo4*	8	18.870	0.013
μSsa407	13	41.642	***	*μOgo4*	8	30.412	***	*μOts3*	8	16.249	0.024
μOcl1	12	28.894	**	*μOki23*	12	28.166	***	*μOts100*	14	24.636	0.027
μOne13	17	31.797	**	*μOmy77*	14	35.790	***	*μOcl1*	15	24.562	0.042
μOki23	11	25.627	**	*μSsa407*	17	47.666	***	*μOmy7i*	14	22.334	0.058
μOmy7i	12	24.467	**	*μOmy1011*	17	38.789	**	*μOts1*	11	17.626	0.072
μOts100	13	26.439	0.007	*μOne14*	8	23.067	**	*μOki23*	12	16.427	0.160
μOmy1011	15	27.01	0.017	*μOts1*	10	25.219	**	*μOmy77*	15	18.466	0.231
μOts4	7	13.741	0.038	*μSsa408*	18	38.320	**	*μSsa289*	6	7.568	0.253
μOgo4	8	14.774	0.041	*μOne13*	17	31.169	**	*μOgo3*	7	8.508	0.285
μOts3	7	13.563	0.043	*μOts100*	15	29.127	**	*μOts4*	6	6.328	0.391
μSsa408	14	21.601	0.075	*μOmy7i*	12	22.364	0.021	*μSsa408*	18	18.348	0.441
μOne14	8	8.843	0.371	*μOts4*	7	15.727	0.029	*μSsa407*	15	13.661	0.581
μOts1	12	12.480	0.417	*μOts3*	8	13.312	0.062	*μOne14*	8	6.569	0.613
μOgo3	6	5.278	0.540	*μSsa289*	6	8.967	0.174	*μOmy1011*	16	12.034	0.790
μSsa289	5	0.623	0.983	*μOgo3*	6	5.765	0.461	*μOne13*	17	12.747	0.805

D.) Lower E.C. vs. ECNFH

Locus	df	χ^2	*P* - value
μOcl1	12	51.120	***
μSsa407	13	52.920	***
μOmy77	14	39.330	***
μOne14	8	26.660	***
μOgo4	8	20.520	**
μOmy7i	11	25.280	**
μOne13	14	24.810	0.014
μOki23	11	21.100	0.022
μOmy1011	16	26.820	0.028
μSsa408	15	23.410	0.062
μOts3	7	11.810	0.083
μOts100	14	16.770	0.257
μOts4	7	7.780	0.370
μOts1	11	10.070	0.548
μOgo3	5	3.490	0.669
μSsa289	5	2.740	0.773

E.) Lower E.C. vs. Adult NOR

Locus	df	χ^2	*P* - value
μOmy7i	13	21.320	0.029
μOcl1	12	20.109	0.042
μSsa407	14	19.760	0.072
μOmy77	14	18.630	0.149
μOne14	8	11.200	0.157
μOgo4	6	9.190	0.160
μSsa408	15	13.520	0.607
μOts4	6	4.710	0.621
μOki23	11	9.060	0.657
μOgo3	5	3.450	0.683
μOts3	8	5.750	0.733
μOts100	13	9.410	0.815
μSsa289	5	2.610	0.844
μOmy1011	14	9.380	0.877
μOne13	14	8.760	0.901
μOts1	11	5.920	0.916

F.) Upper E.C. vs. ECNFH

Locus	df	χ^2	*P* - value
μOgo3	18	48.980	***
μOgo4	9	34.880	***
μSsa289	13	66.170	***
μOts1	13	40.310	***
μOne14	14	43.650	***
μOts3	16	47.070	***
μOki23	17	59.380	***
μOmy1011	19	36.910	**
μOcl1	15	30.870	**
μSsa408	7	17.940	0.008
μOts100	8	18.000	0.016
μOne13	13	24.710	0.019
μOmy77	8	15.410	0.041
μSsa407	11	17.550	0.076
μOts4	7	10.470	0.128
μOmy7i	5	5.910	0.320

G.) Upper E.C. vs. Adult NOR

Locus	df	χ^2	*P* - value
μOmy7i	14	32.925	**
μOcl1	13	26.794	0.014
μOki23	13	23.606	0.028
μOts4	6	12.870	0.042
μOgo4	7	13.597	0.059
μOmy1011	16	24.415	0.074
μOmy77	14	21.721	0.084
μOts1	12	18.479	0.097
μOgo3	8	12.230	0.133
μOne14	9	12.990	0.156
μOts100	15	17.297	0.300
μSsa407	16	17.492	0.350
μSsa289	5	5.632	0.364
μSsa408	16	16.045	0.458
μOne13	19	17.752	0.550
μOts3	8	5.701	0.704

Table 3. Assignment test results: values in the "baseline" columns are from the jackknife re-sampling assignment procedure. The ECNFH smolts represent HAT genotypes, and adults sampled at the Eagle Creek lower ladder represent NOR genotypes (bold Italics indicate correct assignments). The adult NOR group (c) was designated as the critical population. Individuals from each Eagle Creek juvenile sample collection were treated as fish of "unknown" origin, and assigned to a group in the baseline. The symbol (*) indicates the LOD at which 95% confidence of correct assignment was observed, and (new!) is the number of individuals with at least one allele <u>not</u> found in the baseline.

A.

	Baseline Groups		"unknown" samples (2006)		
"WHICHRUN" Statistic[1]	ECNFH (n = 104)	NOR[C] (n = 71)	N. Fork E.C. (n = 69)	Upper E.C. (n = 94)	Lower E.C. (n = 29)
<u>NOR assignments</u>					
Individuals (#)	9	***54***	54	76	24
% (LOD > 0)	8.7	***76.1***	79.3	80.9	82.8
% (LOD > 1.39)*	--	***53.5***	58.0	62.8	69.0
(new!)	--	--	23	16	5
<u>HOR assignments</u>					
Individuals (#)	***95***	17	15	18	5
% (LOD < 0)	***91.3***	23.9	21.7	19.1	17.2
% (LOD < -1.87)*	***54.8***	--	14.5	8.5	6.9
(new!)	--	--	1	2	0

B.

	Baseline Groups		"unknown" samples (2005-2006)		
"WHICHRUN" Statistic[1]	ECNFH (n = 104)	NOR[C] (n = 71)	N. Fork E.C. (n = 121)	Upper E.C. (n = 144)	Lower E.C. (n = 79)
<u>NOR assignments</u>					
Individuals (#)	9	***54***	88	118	71
% (LOD > 0)	8.7	***76.1***	72.7	81.9	89.9
% (LOD > 1.39)*	--	***53.5***	33.1	41.0	74.7
(new!)	--	--	36	28	24
<u>HOR assignments</u>					
Individuals (#)	***95***	17	33	26	8
% (LOD < 0)	***91.3***	23.9	27.3	18.1	10.1
% (LOD < -1.87)*	***54.8***	--	13.2	6.3	3.8
(new!)	--	--	5	4	0

Figure 1. Map of inclusive tributaries, reaches, and genetic sampling locations within Eagle Creek in the Clackamas Basin.

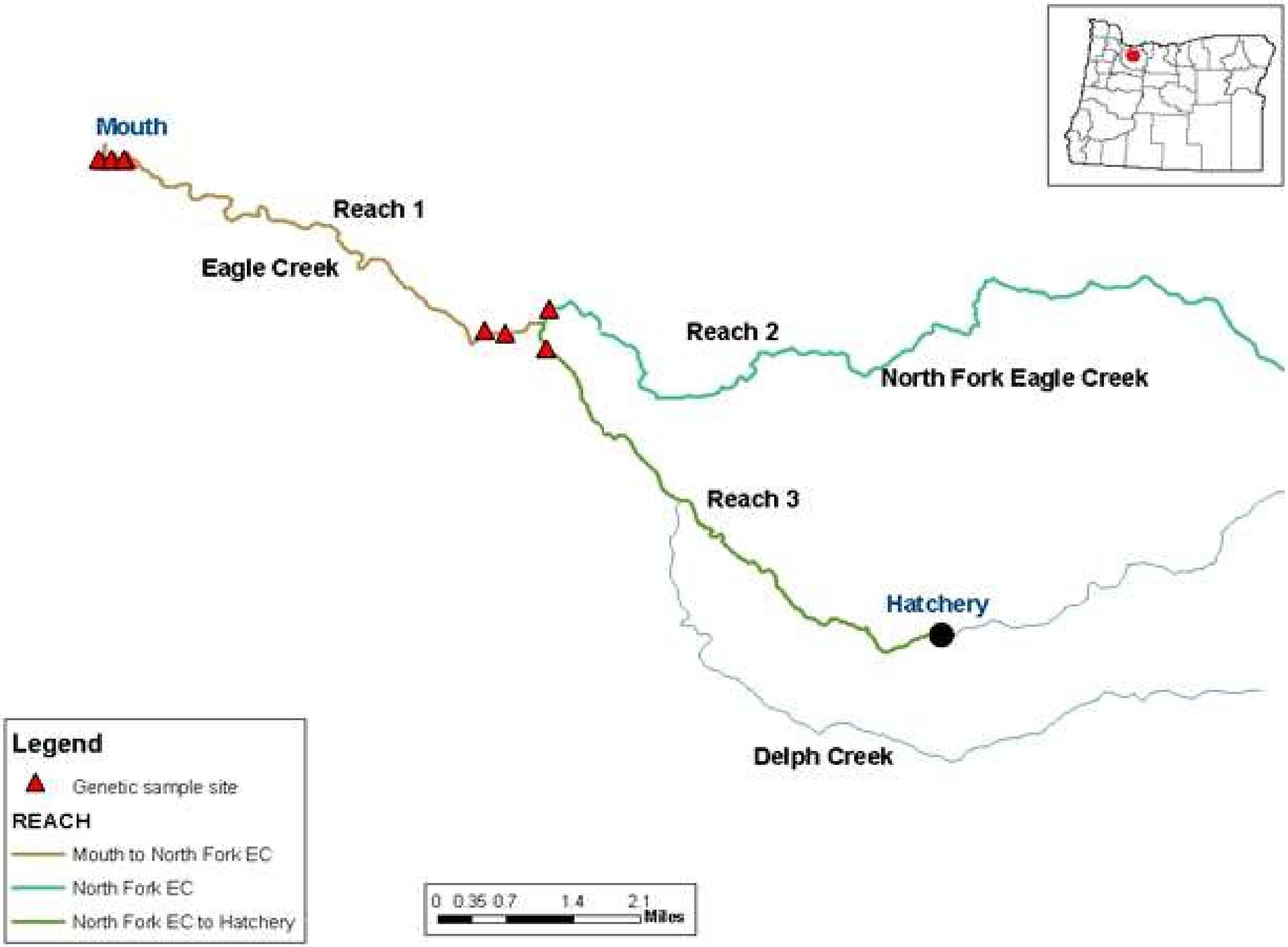


Figure 2. Histogram of allelic richness: values for allelic richness among Eagle Creek-NFH smolts and all natural-origin groups have been scaled to a common sample size using the rarefaction procedure in FSTAT (Goudet 1995). Randomized re-sampling among groups (5000 replicates) indicates a significant difference (*P* = 0.04) in allelic richness between HAT and NOR groups.

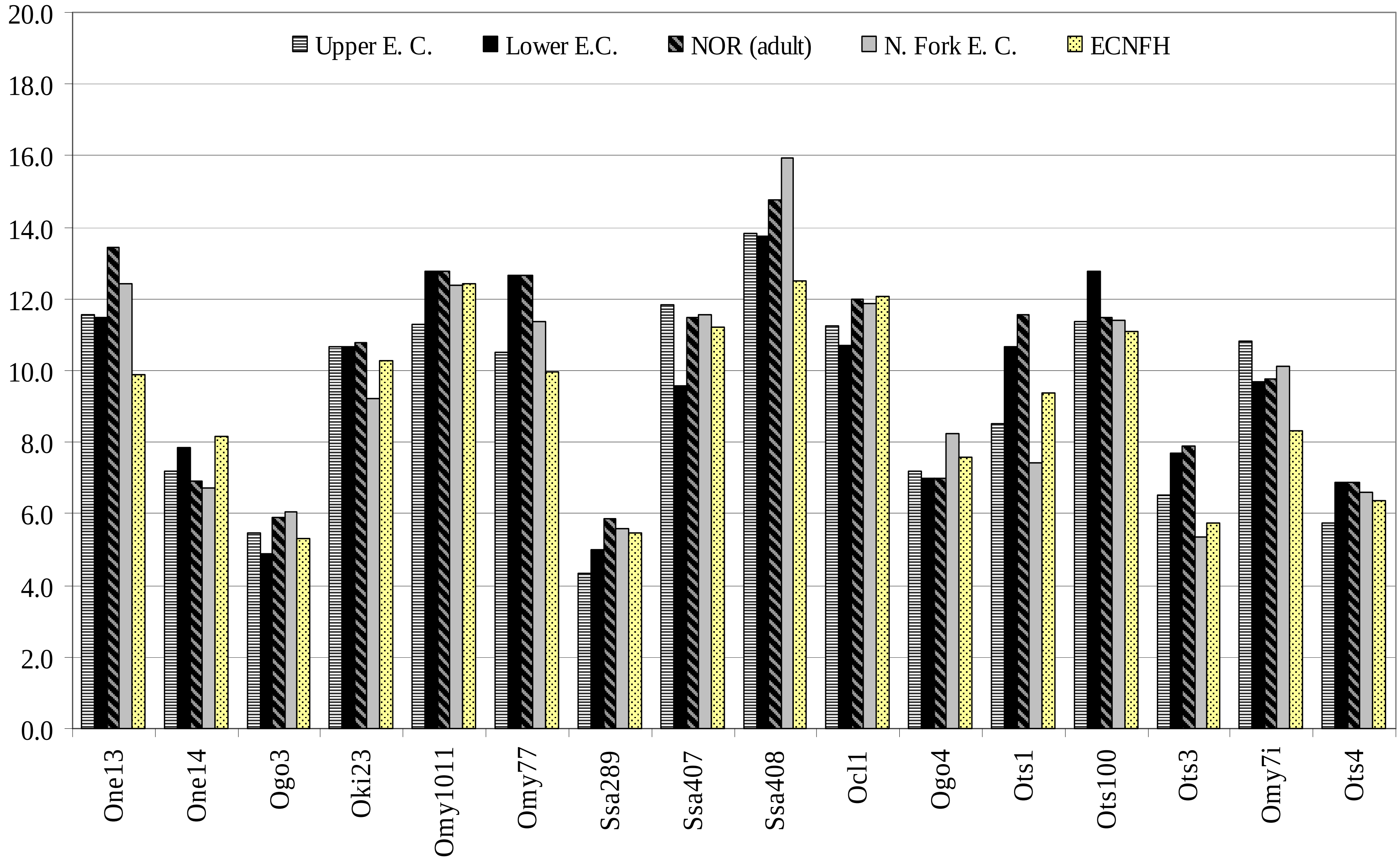


Figure 3. Un-rooted neighbor-joining tree: The phylogram topology was constructed from pairwise genetic (chord) distance measurements (Cavalli-Sforza & Edwards 1967), and includes both the 2005 and 2006 Eagle Creek steelhead collections. Bootstrap support among 1000 replicate data sets is shown between branch nodes, indicates concordance among loci for each branch in the topology.

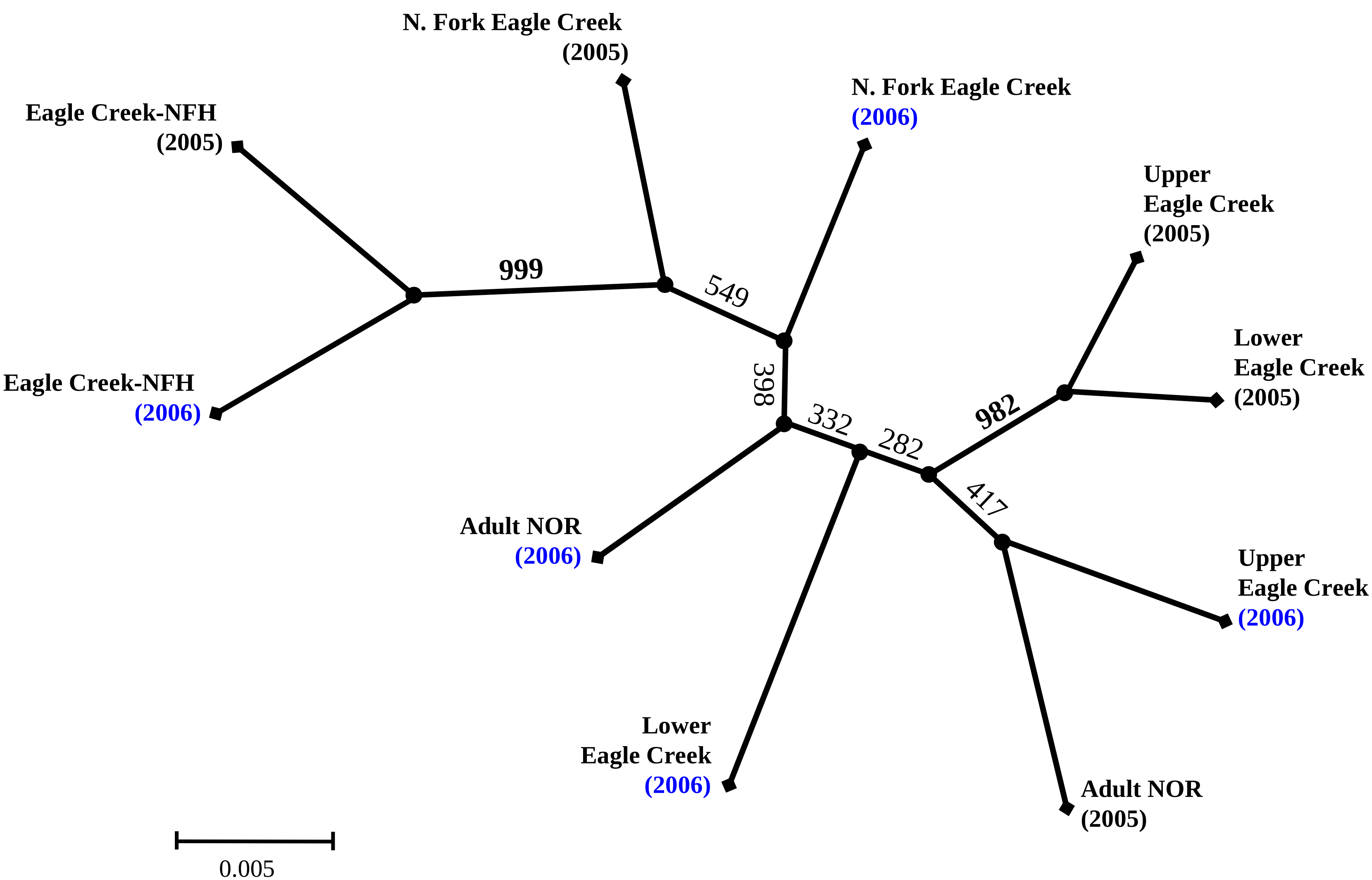
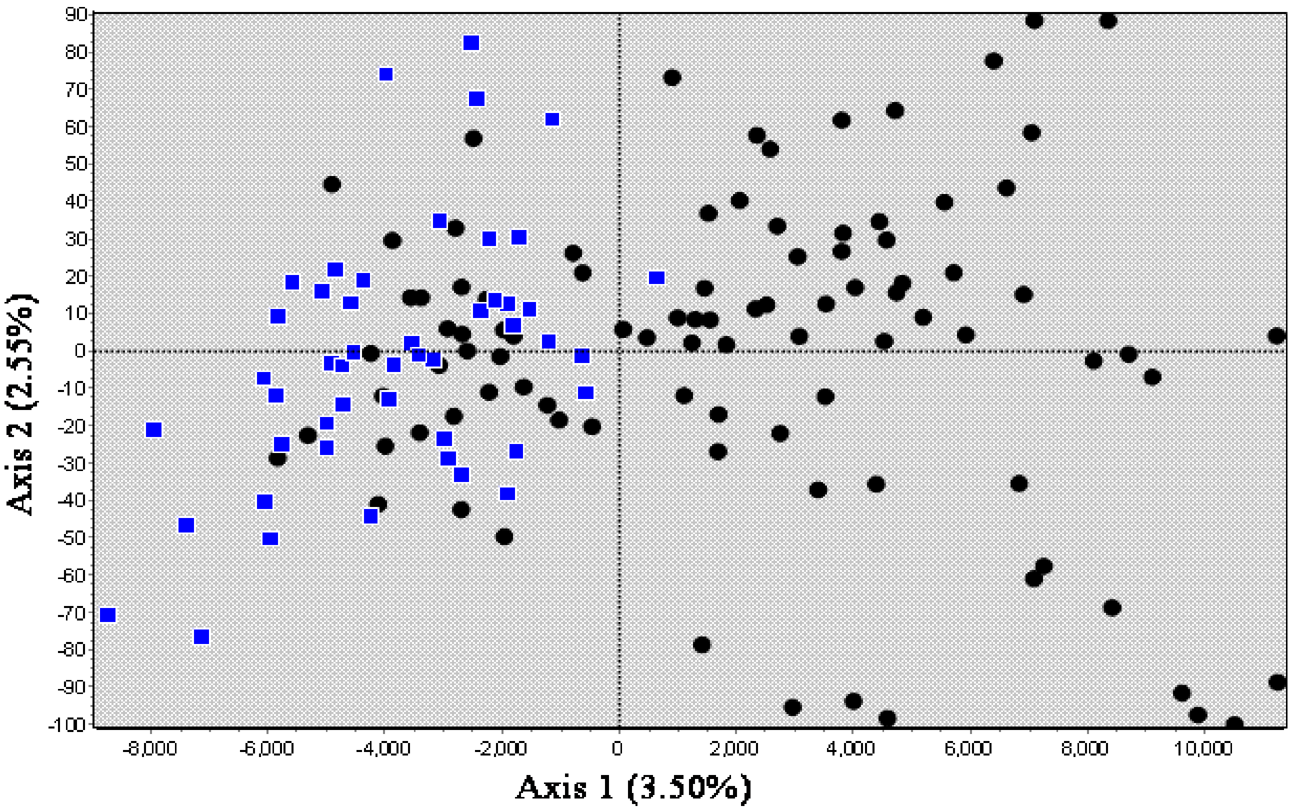
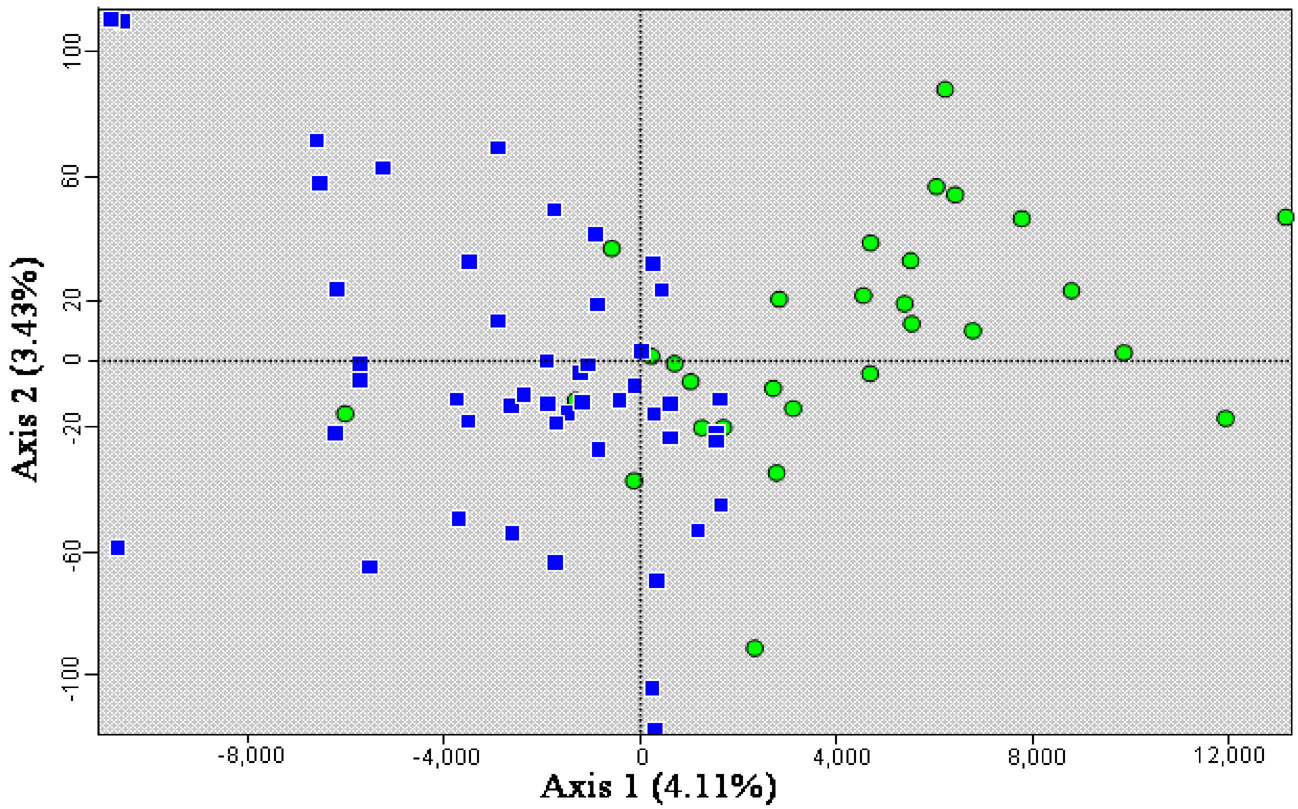


Figure 4. 2-dimensional factorial correspondence plots. Unique variance is identified on the axes, indicating factors with the greatest difference between groups (maximum variability). The 2006 Eagle Creek-NFH steelhead group (blue squares) is shown in relation to 2006 NOR steelhead groups (plots A-D). The last plot (E) describes the relationship among all NOR groups.

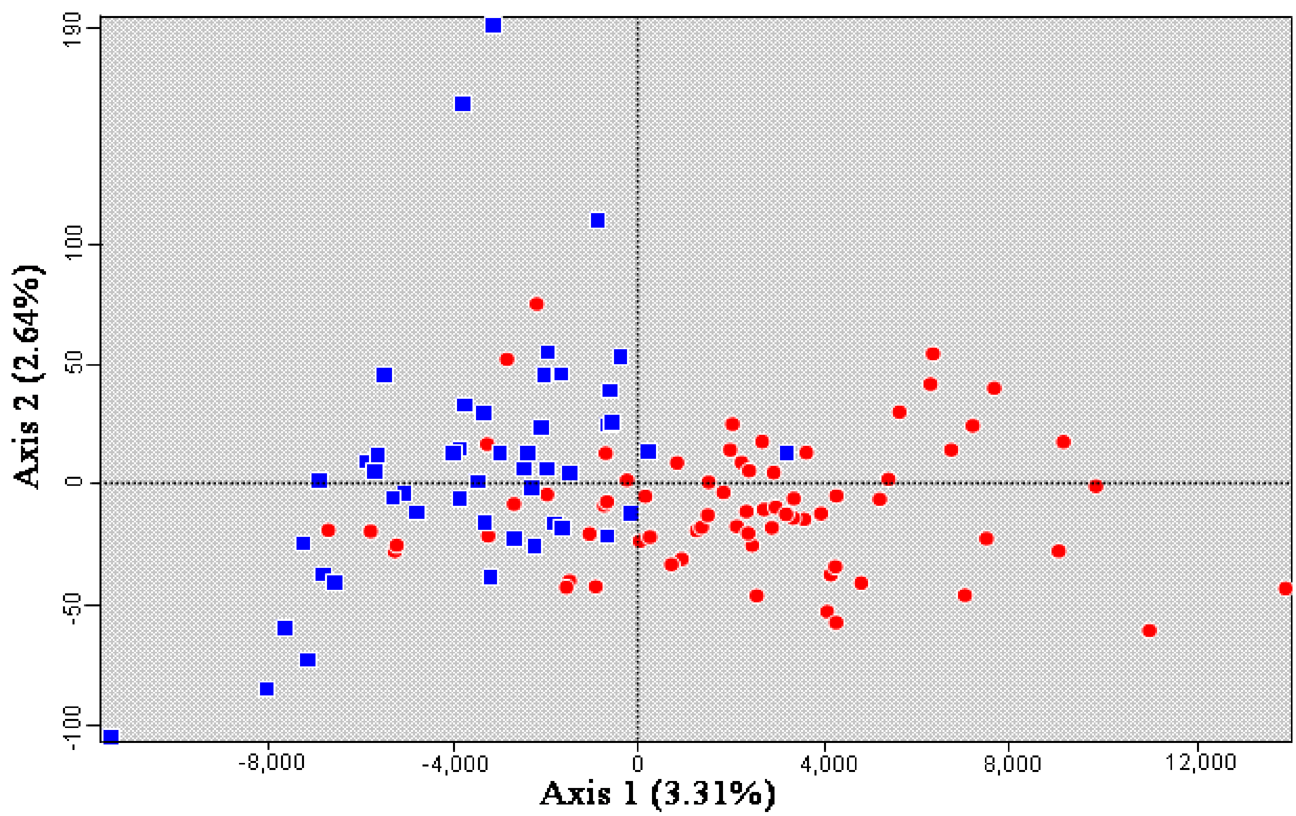
A.) upper Eagle Creek group ●



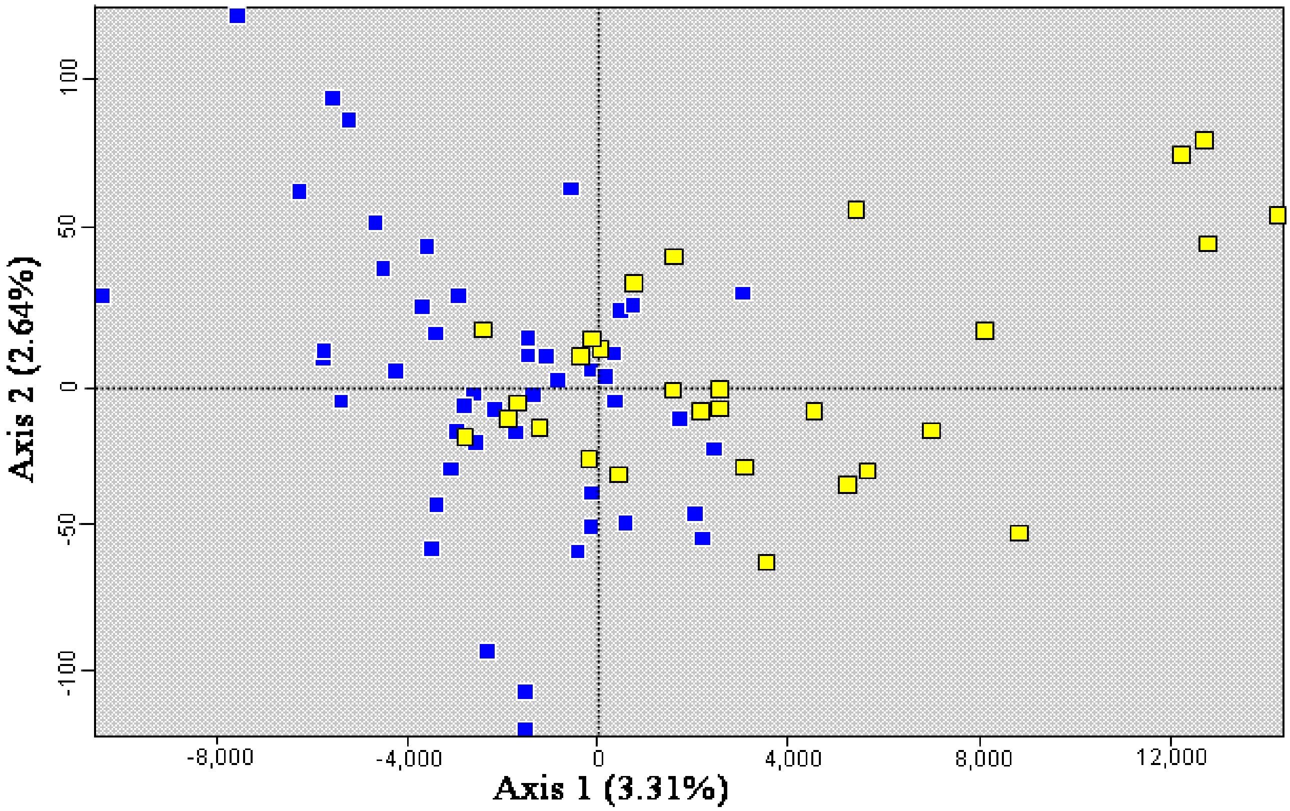
B.) lower Eagle Creek group



B.) N. Fork Eagle Creek group



D.) adult NOR group



E.)

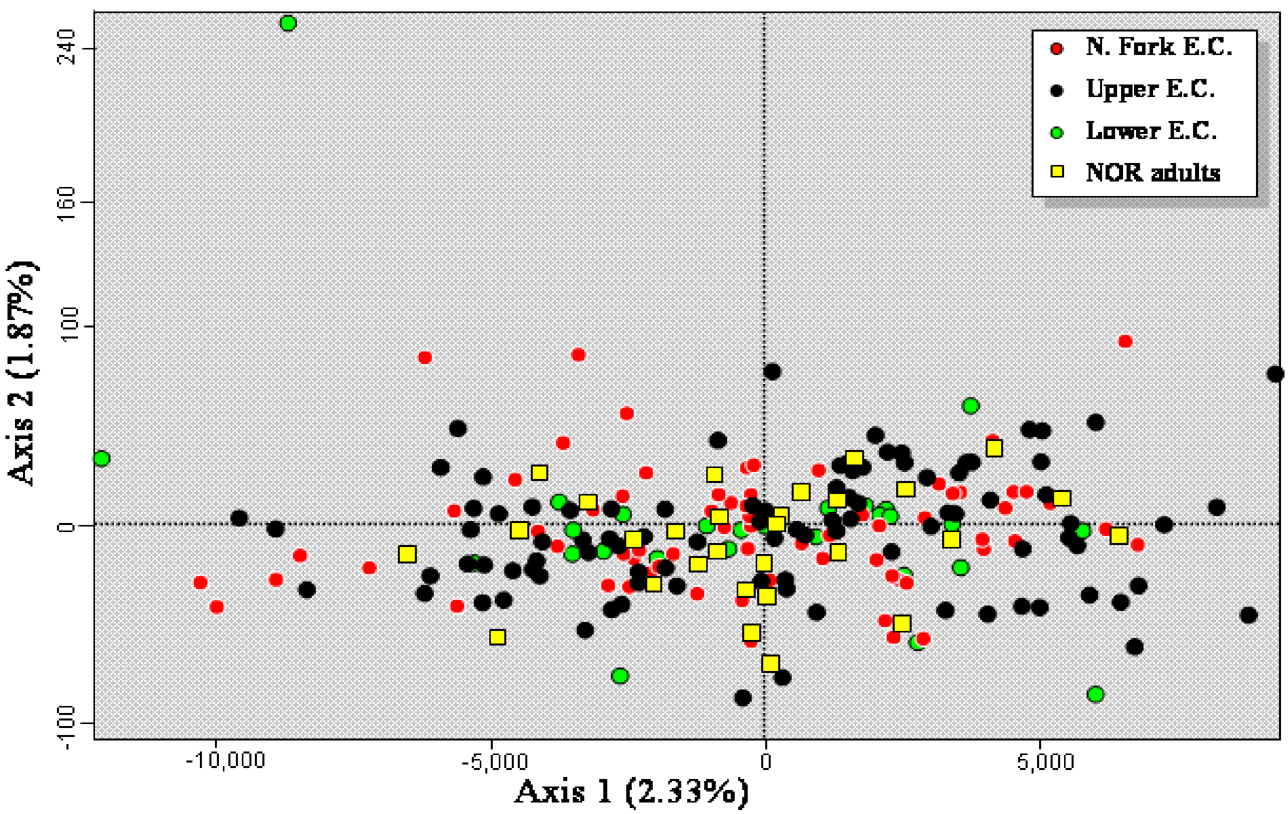


Figure 5. Distribution of assignment stringency (LOD) values from the baseline jack-knife procedure. A LOD > 0 indicates genotypic similarity to the adult NOR group (critical), and a LOD < 0 indicates genotypic similarity to ECNFH. Assignment stringency increases logarithmically, where LOD = 1 indicates a 10 fold greater likelihood of natural origin, and conversely LOD = -1 indicates a 10 fold greater likelihood of hatchery origin. Individuals scoring above LOD = 1.6 were all of natural origin, and individuals scoring below LOD = -2.8 were all of hatchery origin. Dotted lines correspond to the 95% confidence level; LOD < -1.87 for HAT, and LOD > 1.39 for NOR.

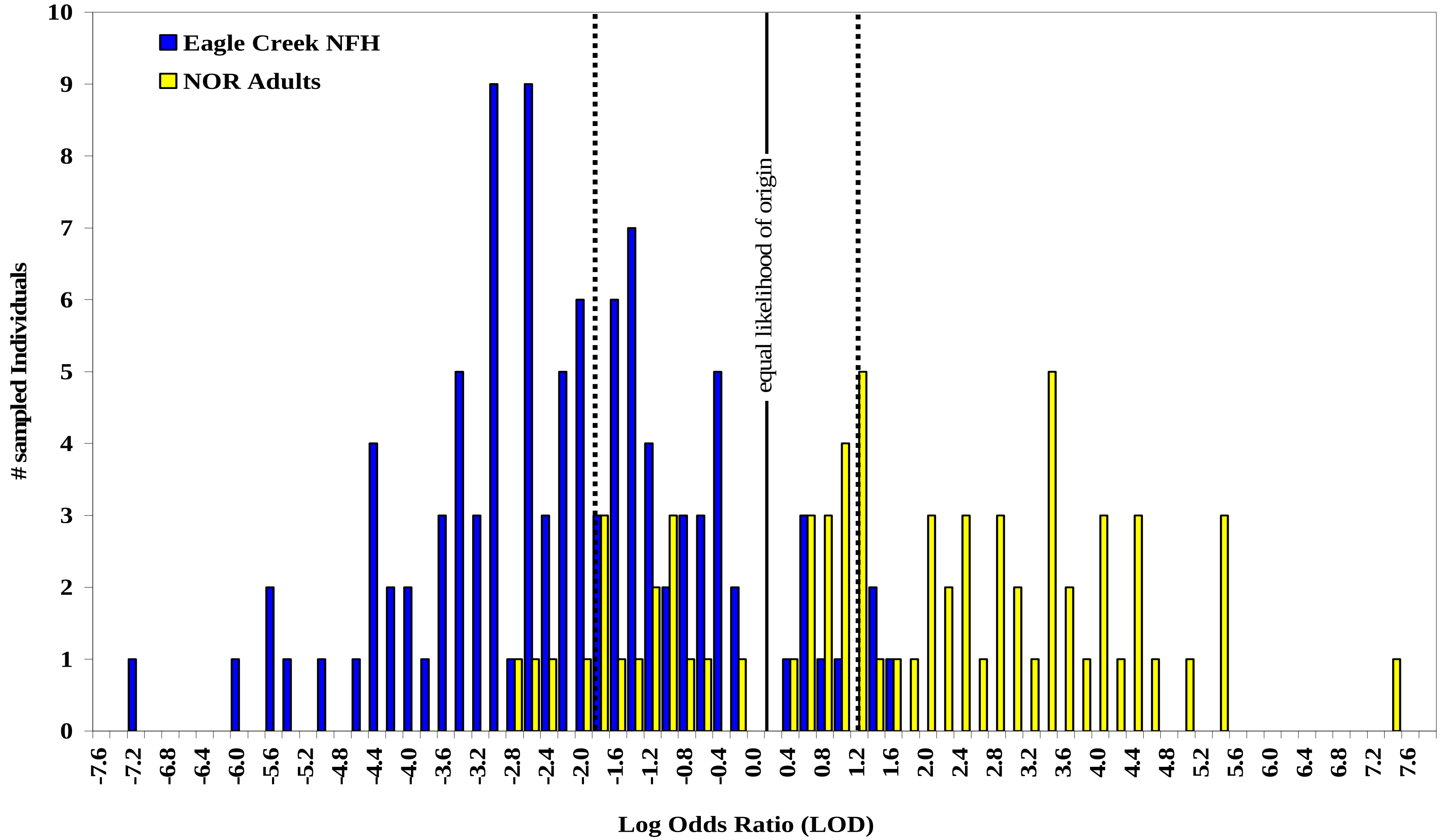
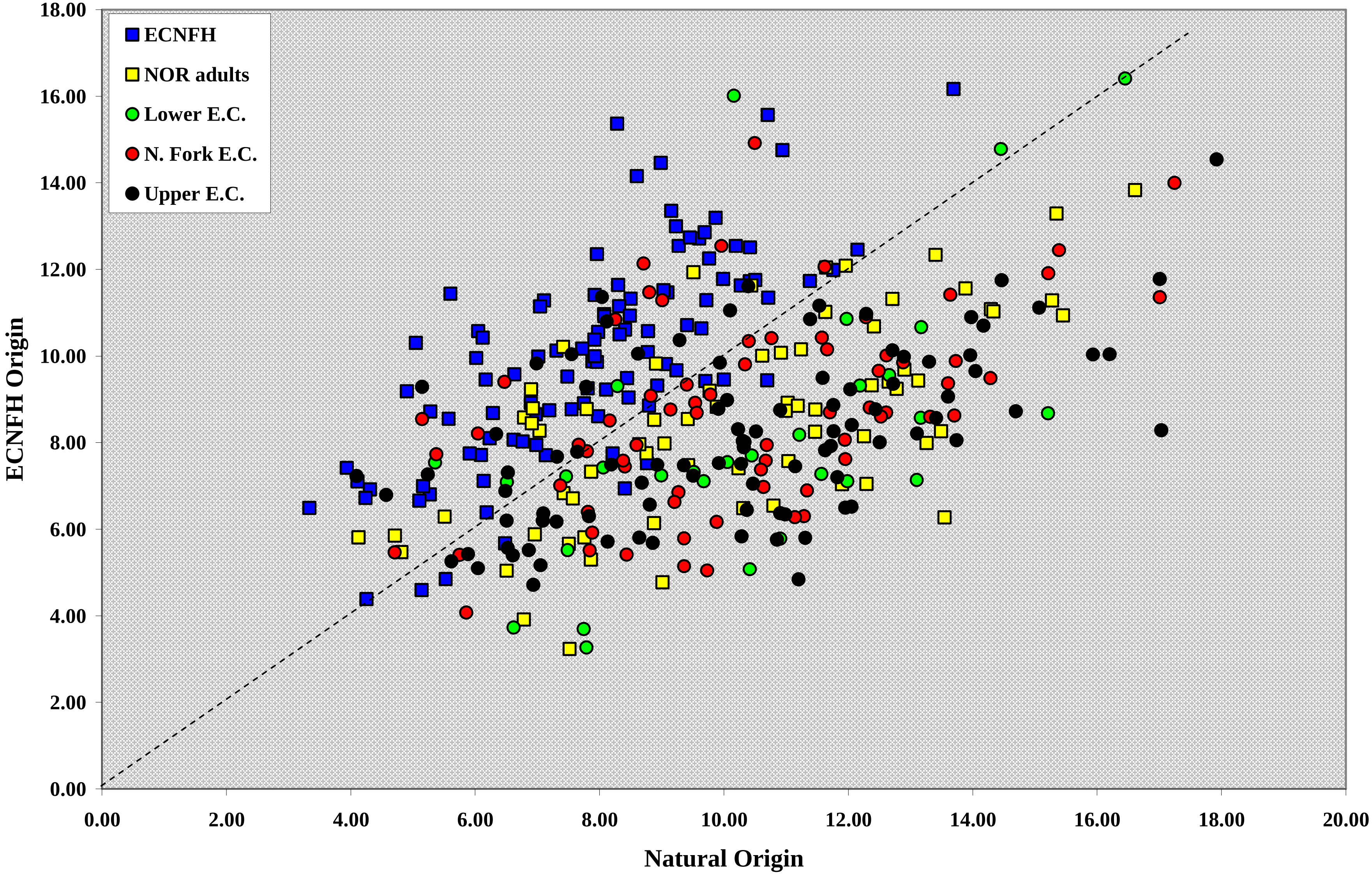


Figure 6. Population assignment plot: coordinates are assignment likelihood values (log-transformed) generated in the program WHICHRUN for all 2006 samples, where the dotted line delineates equal likelihood of NOR or HAT origin.



Appendix 1. 2006 sample collections with biological data recorded by CRFPO personnel. The following samples were excluded from all analyses due to incomplete genotypic data: 384038, 664012, 664023, 664037, 664047. Reach designates the five sample locations: N. fork E. C. is North Fork Eagle Creek, ECNFH is Eagle Creek National Fish Hatchery, Upper E. C. is the stream area between ECNFH & N. Fork E. C., adult NOR are from the lower ladder, and the Lower E.C. section is the stream area between N. Fork E. C. and the confluence of Eagle Creek and the Clackamas River. The last column indicates the LOD score generated in WHICHRUN, used to assign each individual to either HAT or NOR origin (Table 3, Figures 5, 6). F1-hybrid individuals (*O. mykiss-O. clarki*), and cutthroat trout that were originally (phenotypically) identified as rainbow trout were excluded from analyses; these are also indicated in the LOD column.

Reach	Date	Sample ID	LH stage	Length (mm)	Weight (kg)	LOD
ECNFH	7/25/2006	666002	juvenile	78	4.7	0.46
ECNFH	7/25/2006	666003	juvenile	74	4.4	-1.32
ECNFH	7/25/2006	666004	juvenile	72	3.7	-3.16
ECNFH	7/25/2006	666005	juvenile	73	4.4	-0.74
ECNFH	7/25/2006	666006	juvenile	69	3.4	-2.49
ECNFH	7/25/2006	666007	juvenile	68	3.7	-3.77
ECNFH	7/25/2006	666008	juvenile	73	4.3	-1.25
ECNFH	7/25/2006	666009	juvenile	78	4.9	-1.68
ECNFH	7/25/2006	666010	juvenile	75	4.4	-1.90
ECNFH	7/25/2006	666011	juvenile	65	3	-1.79
ECNFH	7/25/2006	666012	juvenile	68	3.2	1.47
ECNFH	7/25/2006	666013	juvenile	73	2.7	-3.28
ECNFH	7/25/2006	666014	juvenile	80	5	1.26
ECNFH	7/25/2006	666015	juvenile	63	2.6	-0.06
ECNFH	7/25/2006	666016	juvenile	67	3	-1.80
ECNFH	7/25/2006	666017	juvenile	74	4.8	-2.48
ECNFH	7/25/2006	666018	juvenile	79	5.8	-2.61
ECNFH	7/25/2006	666019	juvenile	70	3.9	-0.57
ECNFH	7/25/2006	666020	juvenile	71	4.2	-0.97
ECNFH	7/25/2006	666021	juvenile	68	3.3	-2.44
ECNFH	7/25/2006	666022	juvenile	81	6.1	-1.44
ECNFH	7/25/2006	666023	juvenile	63	2.8	-1.83
ECNFH	7/25/2006	666024	juvenile	72	3.7	-0.38
ECNFH	7/25/2006	666025	juvenile	63	2.7	-0.62
ECNFH	7/25/2006	666026	juvenile	73	3.9	-0.31
ECNFH	7/25/2006	666027	juvenile	69	3	-3.32
ECNFH	7/25/2006	666028	juvenile	64	2.6	-2.18
ECNFH	7/25/2006	666029	juvenile	65	3..2	-2.48
ECNFH	7/25/2006	666030	juvenile	69	3.3	-1.55
ECNFH	7/25/2006	666031	juvenile	75	4.2	-4.28

ECNFH	7/25/2006	666032	juvenile	63	2.7	-1.16
ECNFH	7/25/2006	666033	juvenile	66	2.8	-4.10
ECNFH	7/25/2006	666034	juvenile	75	5	-2.04
ECNFH	7/25/2006	666035	juvenile	90	7.4	-3.48
ECNFH	7/25/2006	666036	juvenile	76	4.4	-1.44
ECNFH	7/25/2006	666037	juvenile	83	6	-1.11
ECNFH	7/25/2006	666038	juvenile	73	4.2	-0.22
ECNFH	7/25/2006	666039	juvenile	71	3.5	-0.43
ECNFH	7/25/2006	666040	juvenile	65	2.3	-5.25
ECNFH	7/25/2006	666041	juvenile	79	4.5	-1.26
ECNFH	7/25/2006	666042	juvenile	76	4.5	0.55
ECNFH	7/25/2006	666043	juvenile	74	4.4	-2.07
ECNFH	7/25/2006	666044	juvenile	69	3.1	0.29
ECNFH	7/25/2006	666045	juvenile	85	7.5	-1.35
ECNFH	7/25/2006	666046	juvenile	74	4.7	-2.94
ECNFH	7/25/2006	666047	juvenile	75	5.1	*O. clarki*
ECNFH	7/25/2006	666048	juvenile	88	5.9	-3.16
ECNFH	7/25/2006	666049	juvenile	82	5.2	-5.47
ECNFH	7/25/2006	666050	juvenile	77	5	-2.40
Adult NOR	2/8/2006	383009	adult	610	---	-2.42
Adult NOR	2/8/2006	383010	adult	660	---	2.34
Adult NOR	2/14/2006	383011	adult	780	---	-0.65
Adult NOR	3/7/2006	383012	adult	660	---	2.74
Adult NOR	3/7/2006	383013	adult	820	---	-1.18
Adult NOR	3/7/2006	383014	adult	760	---	4.29
Adult NOR	3/14/2006	383015	adult	560	---	2.78
Adult NOR	3/23/2006	383016	adult	830	---	0.68
Adult NOR	3/23/2006	383017	adult	730	---	1.47
Adult NOR	3/23/2006	383018	adult	550	---	-0.12
Adult NOR	3/23/2006	383019	adult	490	---	-2.33
Adult NOR	3/29/2006	383020	adult	820	---	-1.54
Adult NOR	3/29/2006	383021	adult	770	---	2.87
Adult NOR	3/29/2006	383022	adult	740	---	1.07
Adult NOR	3/29/2006	383023	adult	620	---	0.54
Adult NOR	4/4/2006	383034	adult	710	---	0.84
Adult NOR	4/4/2006	383035	adult	740	---	3.22
Adult NOR	4/4/2006	383036	adult	620	---	1.39
Adult NOR	4/4/2006	383037	adult	600	---	1.08
Adult NOR	4/4/2006	383038	adult	740	---	0.62
Adult NOR	4/12/2006	383050	adult	730	---	1.09
Adult NOR	4/21/2006	383052	adult	640	---	4.00
Adult NOR	4/26/2006	383084	adult	840	---	2.57
Adult NOR	4/26/2006	383085	adult	700	---	5.27
Adult NOR	4/26/2006	383086	adult	610	---	3.31
Adult NOR	4/26/2006	383087	adult	690	---	2.06

Adult NOR	5/4/2006	664073	Adult	540	---	4.52
Adult NOR	5/5/2006	664076	Adult	630	---	2.26
Adult NOR	5/6/2006	664077	Adult	800	---	1.85
Lower E.C.	7/13/2006	664069	juvenile	120	---	1.97
Lower E.C.	8/3/2006	666060	juvenile	47	---	3.03
Lower E.C.	8/3/2006	666061	juvenile	53	---	5.34
Lower E.C.	8/3/2006	666062	juvenile	100	---	2.51
Lower E.C.	8/3/2006	666063	juvenile	110	---	2.89
Lower E.C.	8/3/2006	666064	juvenile	86	---	-1.02
Lower E.C.	8/3/2006	666065	juvenile	138	---	-0.33
Lower E.C.	8/3/2006	666066	juvenile	73	---	2.20
Lower E.C.	8/3/2006	666067	juvenile	138	---	-0.58
Lower E.C.	8/3/2006	666068	juvenile	66	---	-2.18
Lower E.C.	8/3/2006	666069	juvenile	45	---	5.12
Lower E.C.	8/3/2006	666051	juvenile	61	---	4.88
Lower E.C.	8/3/2006	666052	juvenile	67	---	4.59
Lower E.C.	8/3/2006	666055	juvenile	125	---	5.97
Lower E.C.	8/3/2006	666056	juvenile	73	---	3.11
Lower E.C.	8/3/2006	666057	juvenile	51	---	2.75
Lower E.C.	8/3/2006	666058	juvenile	61	---	4.52
Lower E.C.	6/27/2006	664063	juvenile	115	16	2.50
Lower E.C.	6/27/2006	664064	juvenile	141	32	0.04
Lower E.C.	6/27/2006	664065	juvenile	135	30.9	2.88
Lower E.C.	6/27/2006	664066	juvenile	115	21.2	6.54
Lower E.C.	6/27/2006	664067	juvenile	128	26.7	4.05
Lower E.C.	6/27/2006	664068	juvenile	125	23.6	0.25
Lower E.C.	6/27/2006	664060	juvenile	125	28.8	1.12
Lower E.C.	6/27/2006	664061	juvenile	76	4.9	0.64
Lower E.C.	6/27/2006	664062	juvenile	129	27.8	1.75
Lower E.C.	8/3/2006	666053	smolt	170	---	2.57
Lower E.C.	8/3/2006	666054	smolt	163	---	-5.85
Lower E.C.	8/3/2006	666059	smolt	163	---	4.30
N.Fork E.C.	4/13/2006	384013	Juvenile	97	10.1	3.57
N.Fork E.C.	4/25/2006	384014	Juvenile	91	8.1	2.32
N.Fork E.C.	4/25/2006	384015	Juvenile	95	9.1	0.53
N.Fork E.C.	4/25/2006	384016	Juvenile	89	7.1	-0.26
N.Fork E.C.	4/25/2006	384017	Juvenile	108	12.3	5.08
N.Fork E.C.	4/25/2006	384018	Juvenile	88	7.3	2.33
N.Fork E.C.	4/25/2006	384019	Juvenile	102	10.9	3.91
N.Fork E.C.	4/25/2006	384021	Juvenile	109	14.3	2.84
N.Fork E.C.	?	384038	Juvenile	108	11.8	PCR failure
N.Fork E.C.	?	384045	Juvenile	125	21.1	4.87
N.Fork E.C.	5/9/2006	384049	Juvenile	81	6.2	3.85
N.Fork E.C.	5/9/2006	384050	Juvenile	110	13.7	4.24
N.Fork E.C.	5/9/2006	384051	Juvenile	91	7.9	hybrid

N.Fork E.C.	5/9/2006	384052	Juvenile	93	8.1	2.60
N.Fork E.C.	5/9/2006	384055	Juvenile	106	12.8	-0.76
N.Fork E.C.	5/9/2006	384056	Juvenile	106	12.6	-0.29
N.Fork E.C.	5/16/2006	384065	Juvenile	120	18.2	0.36
N.Fork E.C.	5/16/2006	384066	Juvenile	101	9.8	-2.36
N.Fork E.C.	5/16/2006	384067	Juvenile	99	9.1	hybrid
N.Fork E.C.	5/16/2006	384068	Juvenile	117	16.8	-2.16
N.Fork E.C.	5/25/2006	384070	Juvenile	99	---	1.78
N.Fork E.C.	5/25/2006	384071	Juvenile	102	---	3.92
N.Fork E.C.	5/25/2006	384072	Juvenile	91	---	3.25
N.Fork E.C.	5/29/2006	384073	Juvenile	118	---	0.97
N.Fork E.C.	5/29/2006	384074	Juvenile	104	---	1.42
N.Fork E.C.	6/1/2006	384075	Juvenile	111	16.8	0.79
N.Fork E.C.	6/1/2006	384076	Juvenile	95	10.4	0.06
N.Fork E.C.	6/1/2006	384077	Juvenile	109	14.5	3.01
N.Fork E.C.	6/1/2006	384078	Juvenile	110	16.6	2.23
N.Fork E.C.	6/1/2006	384079	Juvenile	96	9.8	0.07
N.Fork E.C.	6/1/2006	384080	Juvenile	112	16.9	3.66
N.Fork E.C.	4/10/2006	384001	SMOLT	133	24.4	0.65
N.Fork E.C.	4/10/2006	384003	SMOLT	135	26.9	-0.44
N.Fork E.C.	4/10/2006	384004	SMOLT	175	59.5	-3.40
N.Fork E.C.	4/10/2006	384005	SMOLT	166	45.4	3.54
N.Fork E.C.	4/10/2006	384006	SMOLT	163	46.1	-2.28
N.Fork E.C.	4/12/2006	384007	SMOLT	180	61.4	-2.58
N.Fork E.C.	4/12/2006	384008	SMOLT	190	60.7	-0.34
N.Fork E.C.	4/12/2006	384009	SMOLT	151	36.4	1.39
N.Fork E.C.	4/12/2006	384010	SMOLT	155	40.7	-3.42
N.Fork E.C.	4/12/2006	384011	SMOLT	175	57.1	0.36
N.Fork E.C.	4/25/2006	384020	SMOLT	155	36.8	4.99
N.Fork E.C.	4/25/2006	384022	SMOLT	163	45.4	0.00
N.Fork E.C.	4/27/2006	384024	SMOLT	182	56.3	0.38
N.Fork E.C.	4/27/2006	384025	SMOLT	159	36.6	2.42
N.Fork E.C.	4/27/2006	384026	SMOLT	178	53.2	4.80
N.Fork E.C.	4/27/2006	384027	SMOLT	170	46	-2.93
N.Fork E.C.	4/27/2006	384028	SMOLT	154	36	3.03
N.Fork E.C.	4/27/2006	384029	SMOLT	138	27	4.22
N.Fork E.C.	4/27/2006	384030	SMOLT	172	49	1.51
N.Fork E.C.	4/27/2006	384031	SMOLT	152	30.1	-2.67
N.Fork E.C.	?	384034	SMOLT	161	40.7	2.74
N.Fork E.C.	?	384035	SMOLT	140	36.4	4.33
N.Fork E.C.	?	384036	SMOLT	166	44.6	3.72
N.Fork E.C.	?	384039	SMOLT	193	11.8	1.97
N.Fork E.C.	?	384040	SMOLT	146	30.8	0.34
N.Fork E.C.	?	384041	SMOLT	153	31.3	4.72
N.Fork E.C.	?	384042	SMOLT	153	35.4	-2.59

N.Fork E.C.	?	384043	SMOLT	156	38.3	4.69
N.Fork E.C.	?	384044	SMOLT	148	33.5	3.88
N.Fork E.C.	?	384046	SMOLT	150	28.7	2.57
N.Fork E.C.	5/9/2006	384047	SMOLT	160	38.9	3.31
N.Fork E.C.	5/9/2006	384048	SMOLT	192	59.7	hybrid
N.Fork E.C.	5/9/2006	384053	SMOLT	165	45.4	5.66
N.Fork E.C.	5/9/2006	384054	SMOLT	155	40.2	1.15
N.Fork E.C.	5/10/2006	384057	SMOLT	168	45.7	4.45
N.Fork E.C.	5/10/2006	384058	SMOLT	156	33	0.62
N.Fork E.C.	5/10/2006	384059	SMOLT	141	29.4	0.87
N.Fork E.C.	5/10/2006	384060	SMOLT	133	25.3	2.95
N.Fork E.C.	5/10/2006	384061	SMOLT	163	50	3.09
N.Fork E.C.	5/10/2006	384062	SMOLT	156	35.5	3.02
N.Fork E.C.	5/16/2006	384063	SMOLT	120	17.1	-4.42
N.Fork E.C.	5/16/2006	384064	SMOLT	138	26.1	3.22
Upper E.C.	3/30/2006	383025	Juvenile	136	27	1.88
Upper E.C.	3/31/2006	383030	Juvenile	104	10.9	3.18
Upper E.C.	3/31/2006	383031	Juvenile	75	4.2	3.42
Upper E.C.	4/4/2006	383032	Juvenile	84	6.2	-2.02
Upper E.C.	4/5/2006	383039	Juvenile	110	13.8	1.07
Upper E.C.	4/5/2006	383040	Juvenile	85	5.6	4.54
Upper E.C.	4/6/2006	383041	Juvenile	75	4.6	5.69
Upper E.C.	4/7/2006	383046	Juvenile	95	9.2	2.10
Upper E.C.	4/7/2006	383047	Juvenile	66	2.7	5.46
Upper E.C.	4/10/2006	383048	Juvenile	93	7.7	3.39
Upper E.C.	4/12/2006	383049	Juvenile	64	---	5.09
Upper E.C.	4/21/2006	383053	Juvenile	61	2.4	5.24
Upper E.C.	4/21/2006	383055	Juvenile	80	5.7	4.90
Upper E.C.	4/21/2006	383056	Juvenile	70	3.6	4.40
Upper E.C.	4/21/2006	383057	Juvenile	63	2.8	1.44
Upper E.C.	4/21/2006	383059	Juvenile	62	2.6	4.54
Upper E.C.	4/21/2006	383061	Juvenile	121	16.8	-2.68
Upper E.C.	4/24/2006	383063	Juvenile	71	4.2	3.80
Upper E.C.	4/24/2006	383064	Juvenile	85	6.5	4.64
Upper E.C.	4/24/2006	383066	Juvenile	68	4	2.81
Upper E.C.	4/24/2006	383067	Juvenile	74	4.7	6.16
Upper E.C.	4/24/2006	383070	Juvenile	100	11.2	0.46
Upper E.C.	4/24/2006	383071	Juvenile	86	7.5	0.70
Upper E.C.	4/24/2006	383072	Juvenile	89	7.6	2.91
Upper E.C.	4/25/2006	383073	Juvenile	69	3	1.13
Upper E.C.	4/25/2006	383076	Juvenile	67	3	1.35
Upper E.C.	4/25/2006	383077	Juvenile	79	4.8	2.39
Upper E.C.	4/25/2006	383078	Juvenile	93	7.5	2.28
Upper E.C.	4/26/2006	383081	Juvenile	167	44.9	0.36
Upper E.C.	5/1/2006	383091	Juvenile	63	8.6	2.27

Upper E.C.	5/1/2006	383092	Juvenile	60	2.6	1.92
Upper E.C.	5/1/2006	383093	Juvenile	77	5.1	8.75
Upper E.C.	5/1/2006	383094	Juvenile	60	2.6	3.48
Upper E.C.	5/4/2006	383100	Juvenile	109	12.6	1.88
Upper E.C.	5/8/2006	664004	juvenile	94	8.6	4.85
Upper E.C.	5/8/2006	664005	juvenile	95	10.2	-2.49
Upper E.C.	5/8/2006	664006	juvenile	85	8.5	2.43
Upper E.C.	5/8/2006	664007	juvenile	69	3.6	3.36
Upper E.C.	5/8/2006	664008	juvenile	79	5.7	3.69
Upper E.C.	5/8/2006	664009	juvenile	105	14.2	3.67
Upper E.C.	5/8/2006	664011	juvenile	76	5.9	-1.86
Upper E.C.	5/8/2006	664012	juvenile	87	7.8	PCR failure
Upper E.C.	5/8/2006	664013	juvenile	95	10.8	-1.08
Upper E.C.	5/8/2006	664014	juvenile	91	9	2.77
Upper E.C.	5/8/2006	664015	juvenile	75	5	3.65
Upper E.C.	5/8/2006	664017	juvenile	104	13.2	-3.32
Upper E.C.	5/8/2006	664018	juvenile	105	12.7	*O. clarki*
Upper E.C.	5/8/2006	664019	juvenile	85	7.6	3.80
Upper E.C.	5/8/2006	664020	juvenile	74	5.2	2.15
Upper E.C.	5/8/2006	664021	juvenile	72	4.8	5.90
Upper E.C.	5/8/2006	664022	juvenile	80	5.8	-1.50
Upper E.C.	5/8/2006	664023	juvenile	61	3	PCR failure
Upper E.C.	5/8/2006	664024	juvenile	98	11.6	-0.36
Upper E.C.	5/8/2006	664025	juvenile	91	9.5	1.61
Upper E.C.	5/8/2006	664027	juvenile	79	5.8	1.32
Upper E.C.	5/8/2006	664028	juvenile	68	3.9	3.94
Upper E.C.	5/8/2006	664029	juvenile	60	2.6	2.90
Upper E.C.	5/8/2006	664030	juvenile	80	6.4	-4.14
Upper E.C.	5/8/2006	664031	juvenile	99	11.7	2.84
Upper E.C.	5/8/2006	664032	juvenile	78	5.4	0.09
Upper E.C.	5/8/2006	664033	juvenile	72	4.3	3.93
Upper E.C.	5/8/2006	664034	juvenile	90	7.9	0.96
Upper E.C.	5/8/2006	664035	juvenile	78	5.5	2.42
Upper E.C.	5/8/2006	664036	juvenile	84	7.5	0.38
Upper E.C.	5/8/2006	664037	juvenile	74	5.3	PCR failure
Upper E.C.	5/8/2006	664046	juvenile	77	5.9	3.44
Upper E.C.	5/8/2006	664047	juvenile	96	10.7	PCR failure
Upper E.C.	5/8/2006	664048	juvenile	73	4.9	2.22
Upper E.C.	5/8/2006	664049	juvenile	75	5.5	6.36
Upper E.C.	5/8/2006	664051	juvenile	82	7.1	4.63
Upper E.C.	5/8/2006	664052	juvenile	85	7.6	1.53
Upper E.C.	5/8/2006	664053	juvenile	76	5.5	2.26
Upper E.C.	5/8/2006	664054	juvenile	77	5.8	0.74
Upper E.C.	5/8/2006	664055	juvenile	60	2.7	2.71
Upper E.C.	5/4/2006	383026	smolt	166	38	0.54

Upper E.C.	3/30/2006	383027	smolt	155	35.9	5.97
Upper E.C.	4/4/2006	383033	Smolt	158	37.7	5.54
Upper E.C.	4/6/2006	383042	smolt	179	47.8	-1.43
Upper E.C.	4/6/2006	383043	smolt	163	36.8	1.13
Upper E.C.	4/6/2006	383044	smolt	179	61.5	-0.78
Upper E.C.	4/21/2006	383054	smolt	170	44.4	2.58
Upper E.C.	4/21/2006	383058	smolt	149	29	-3.13
Upper E.C.	4/21/2006	383060	smolt	180	54.2	2.24
Upper E.C.	4/21/2006	383062	smolt	161	40	4.45
Upper E.C.	4/24/2006	383069	smolt	163	47.2	-0.95
Upper E.C.	4/25/2006	383080	smolt	178	48.9	5.51
Upper E.C.	4/26/2006	383082	smolt	225	108.5	-0.14
Upper E.C.	4/26/2006	383083	smolt	161	39.4	3.96
Upper E.C.	4/27/2006	383088	smolt	201	71.9	1.21
Upper E.C.	4/27/2006	383089	smolt	185	51.7	-0.39
Upper E.C.	5/3/2006	383095	smolt	176	43.6	0.31
Upper E.C.	5/4/2006	383098	smolt	192	64.3	-2.84
Upper E.C.	5/4/2006	383099	smolt	179	52.6	4.50
Upper E.C.	5/8/2006	664001	smolt	209	92.7	-1.22
Upper E.C.	5/8/2006	664002	smolt	170	41.3	-2.22
Upper E.C.	5/8/2006	664003	smolt	179	53.8	0.95
Upper E.C.	5/5/2006	664071	smolt	166	40.4	3.08
Upper E.C.	5/5/2006	664074	smolt	165	39.6	3.50
Upper E.C.	5/6/2006	664075	smolt	195	62.4	0.89